The role of sodium hyaluronate and sodium chondroitin sulphate in the management of bladder disease

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***Abstract*:** Bladder epithelium is not only a simple defence against infections, but it is also a specialized tissue regulating complex bladder functions and playing an active role in the pathogenesis of many bladder diseases. There is strong evidence that different chronic inflammatory bladder diseases, such as recurrent urinary tract infection (UTI), chemical or radiation cystitis and painful bladder syndrome/interstitial cystitis (PBS/IC), can be pathophysiologically linked in the first step of the disease to the loss of the glycosaminoglycan (GAG) mucous layer independently of the original cause of the inflammatory process. The aim of this article is to review the current evidence on the clinic applications of GAGs in urology, with particular emphasis on the therapeutic use of hyaluronic acid (HA) and chondroitin sulphate (CS). A comprehensive electronic literature search was conducted in May 2011 using the Medline database. Three studies supported the decrease of the rate of recurrent UTIs by restoring the GAG layer, showing a significant reduction of UTI rates and a prolonged median time to recurrence after HA intravesical instillations in women with recurrent UTI. We provide higher level evidence by reporting a prospective, randomized, double-blind, placebo-controlled study on the use of intravesical HA and CS in women with recurrent UTIs. A significant reduction of 77% in the UTI rate per patient per year *versus* placebo was observed at the end of the study. Nine studies were published between 2002 and 2011 on the use of HA and CS to treat PBS/IC. Three of them evaluated the use of GAGs bladder instillation to prolong the effects of bladder hydrodistension. In the other six studies the efficacy of HA bladder instillations to reduce symptoms score was assessed. Preliminary studies support data on the role of HA–CS in detrusor overactivity, nonbacterial cystitis and urological malignancies. Few data are available regarding the mode of action of HA–CS or its effectiveness in the management of bladder diseases. The major issue in interpreting the available evidence regarding HA–CS is that most of the reported studies are nonrandomized and without a control arm. HA–CS may be considered for further studies, including randomized, controlled trials with adequate power.

***Keywords*:** urothelium, glycosaminoglycan, hyaluronic acid, sodium chondroitin sulphate

Introduction

Bladder epithelium (BE), also known as 'transitional epithelium' or 'urothelium', is not only a simple defence against infections, but it is also a specialized tissue regulating complex bladder functions and playing an active role in the pathogenesis of many bladder diseases.

Evidence suggests that the urothelium has two main active functions [Arms and Vizzard, 2011; Kanai, 2011; Clemens 2010]: 'afferent' function, which informs the central nervous system on local stimuli and therefore is involved in the control of the micturition reflex, pain reflex, and cardiovascular reflex modulation; 'efferent'

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(or paracrine) function, which is linked to the release of mediators, such as substance P and tachykinins, provoking smooth muscle contraction and local inflammation.

BE is coated by a thick layer of glycosaminoglycans (GAGs) that acts as a nonspecific antiadherence factor and a nonspecific defence mechanism against infection and found in urine irritants (e.g. urea and potassium). GAGs are long, linear and highly negative charged heterogeneous polysaccharides composed of a variable number of repeating disaccharide units.

There are two main types of GAGs: nonsulphated GAGs, that is, hyaluronic acid (HA), and sulphated GAGs, that is, heparan sulphate and heparin, chondroitin sulphate (CS), dermatan sulphate, and keratan sulphate. With the exception of HA, GAGs are usually covalently attached to a protein core, forming an overall structure that is referred to as a proteoglycan [Lilly and Parsons, 1990].

There is strong evidence that different chronic inflammatory bladder diseases, such as recurrent urinary tract infection (UTI), chemical or radiation cystitis, and painful bladder bladder syndrome (PBS)/interstitial cystitis (IC) can be pathophysiologically linked in the first step of the disease to the loss of the GAGs mucous layer independently of the original cause of the inflammatory process [Iavazzo *et al.* 2007].

A cascade of events starting from a GAG injury that fails to heal may lead to chronic bladder epithelial damage and neurogenic inflammation [Geppetti *et al.* 2008].

Various causes, such as autoimmune diseases, chronic bacterial infections, chemicals including Calmette–Guérin exposure, may be considered in the early stage of urothelial GAG loss. The loss of the watertight function of the urothelium would allow both the normal and abnormal (i.e. metabolites of cytotoxic drugs or toxic substances excreted in urine) constituents of urine to come into direct contact with the subepithelial layers causing inflammation and delayed healing of the damaged urothelial layer and GAGs.

The activation of the peptide-containing fibres in the suburothelium is responsible for neuronal hypersensitivity that leads to allodynia with increased frequency, nocturia, urgency and pain during filling.

According to these theories, the early repair of the GAG layer by HA and CS might avoid the chronic evolution of bladder inflammation. The restoration of the GAG layer has recently become the main aim of new therapies for the treatment of chronic cystitis and PBS/IC.

The aim of this article is to review the current evidence on the clinic applications of GAGs in urology, with particular emphasis on the therapeutic use of HA and CS.

A comprehensive electronic literature search was conducted in May 2011 using the Medline database, through either PubMed or Ovid as a search engine, to identify all publications relating to HA, CS, GAG replacement treatment, intravesical administration and bladder disease in urology. Both experimental and clinical research studies were considered. English language articles were included for review, and non-English articles were included if they provided additional, relevant information.

The search was conducted using a free-text protocol that included the following terms: hyaluronic acid, chondroitin sulphate, glycosaminoglycan replacement treatment, intravesical administration and bladder disease.

Recurrent UTIs

UTIs are very common in women and they represent one of the most formidable challenges in clinical practice, given their high prevalence, frequent recurrence and myriad of associated morbidities in the setting of rapidly evolving antimicrobial resistance. According to the European Association of Urology guidelines [Naber *et al.* 2001], recurrent UTIs are defined as at least three episodes of uncomplicated infection documented by urine culture with the isolation of greater than 10^3 colony-forming units/ml. It is estimated that approximately 20% of patients with UTI will develop a second infection within 6 months [Foxman *et al.* 2002; Foxman, 2000].

Low-dose antimicrobial therapy may be used to prevent recurrent UTIs in affected women but it generates antimicrobial resistance and side

effects. Thus, nonantimicrobial prevention strategies are welcomed.

Damage of the urothelial GAGs layer may facilitate bacterial adherence and infection development [Parsons *et al.* 1994].

There is some evidence that suggests HA and CS instillation can be recommended for women with recurrent UTIs (Table 1).

Constandinides and colleagues first supported the decrease in the rate of recurrent UTIs by restoring the GAG layer [Constandinides *et al.* 2004]. They showed a significant reduction in the UTI rates per patient, both in number of infections per year (from 4.3 to 0.3, $p < 0.001$) and prolonged median time to recurrence (from 96 to 498 days, $p < 0.001$) after HA intravesical instillations in 40 women with recurrent UTI.

Lipovac and colleagues evaluated the efficacy of nine HA bladder instillations over 6 months in 20 women with a history of recurrent UTI [Lipovac *et al.* 2007]. Their status was assessed prospectively but compared with a retrospective review of patients' charts. The number of infections per year per patient was significantly reduced (from 4.99 ± 0.92 to 0.56 ± 0.82, $p > 0.001$) and the mean time to recurrence (from 76.7 ± 24.6 to 178.3 ± 25.5 days, $p > 0.001$) was prolonged significantly. Nevertheless 65% of patients were free of recurrences until the end of study (47.6 weeks).

It should be noted that, despite providing encouraging results, no control group was included in both these early studies.

More recently, our group was able to provide a higher level of evidence by reporting a prospective, randomized, double-blind, placebo-controlled study on the use of intravesical HA and CS in women with recurrent UTIs [Damiano *et al.* 2011]. A significant reduction of 77% (mean difference, 95% confidence interval, 73.2–80.8, $p = 0.0002$) in the UTI rate per patient per year *versus* placebo ($-86.6 \pm 47.6\%$ *versus* $-9.6 \pm 24.6\%$) was observed at the end of the study. Moreover, mean time to UTI recurrence was significantly prolonged (185.2 ± 78.7 *versus* 52.7 ± 33.4 days, $p < 0.001$) after treatment with HA–CS compared with placebo. Overall urinary symptoms and quality of life measured by questionnaires significantly improved

Table 1. A comparative analysis of studies on recurrent urinary tract infections.

Study	Study design	Number of patients	Mean age (years)	Hyaluronic acid dose	Regimen	Follow up	Outcome parameters	Results (mean value in days)
Constantinides *et al.* [2004]	PCS	40	35	40 mg	Four weekly plus four monthly	12.4 months	UTI recurrence time; UTI recurrence free	Prolonged (498 *versus* 96); 100%
Lipovac *et al.* [2007]	PCS	20	27	40 mg	Four weekly plus five monthly	47 weeks	Number of infections per patient; Infection rate per patient-year; UTI recurrence time	Reduction (4.99 *versus* 0.56); Improvement (4.99 *versus* 0.56); Prolonged (178 *versus* 76)
Damiano *et al.* [2011]	RCT	57	34	50 ml (hyaluronic acid; 1.6%; chondroitin sulphate 2%)	Four weekly plus five monthly	12 months	UTI rate UTI recurrence time; Quality of life score; Pelvic pain and urinary/frequency questionnaire score	Reduction (–86% *versus* –9.6%); Prolonged (185 *versus* 52); Improvement (78 *versus* 53); Reduction (14 *versus* 10)

PCS, prospective cohort studies; RCT, randomized controlled trial; UTI, urinary tract infection.

compared with placebo. Finally, serious adverse events were not observed in this study.

Overall, in all above mentioned studies, serious adverse events were not observed.

In contrast to traditional antibiotic therapy, which aims at eradicating pathogens, treatment with HA targets bacterial adherence to the bladder mucosa with the presumption that a damaged GAG layer facilitates bacterial adherence and therefore recurrent UTI, and repair of the GAG layer is capable of preventing adherence.

PBS/IC

PBS/IC is a chronic intermittent clinical syndrome characterized by a constellation of symptoms that include bladder/pelvic pain associated with urinary urgency, frequency and dysuria [van de Merwe *et al.* 2008].

It has been estimated that approximately 500,000 individuals in the USA have PBS/IC; the vast majority are adult women, only 10% are men [Jones *et al.* 1997]. It is frequently a diagnosis of exclusion so the average delay in diagnosis is estimated to be 4 years.

The exact aetiology of this disease is still unknown and various hypotheses have been postulated including autoimmune and allergic reactions and occult infection. The most recent one is based on a disorder of the lower urinary dysfunction epithelium [Parsons, 2011]. In other words, the loss of the GAGs 'water-tight' function exposes the bladder submucosa to toxic substances present in urine (normal substances such as Na–K–H–Cl and abnormal substances such as cytotoxic drugs and toxins). Once these irritating substances penetrate the bladder wall, a 'domino-like' mechanism is activated and the bladder fails to repair the damage.

Many drugs have been proposed for the treatment of PBS/IC, many having anti-inflammatory action. Botulin toxin A is also described on the basis of experimental studies that showed the positive effect on visceral pain [Cervigni *et al.* 2008].

Nine studies were published between 2002 and 2011 on the use of HA and CS to treat PBS/IC (Table 2). Three of them evaluated the use of GAGs bladder instillation to prolong the effects of bladder hydrodistention (BH), which represents one of the oldest diagnostic and therapeutic tools for IC. It has been hypothesized that bladder stretching may injure nerve endings in the bladder and thereby reduce pain for reasons that are still unclear. BH provides symptoms relief at 6 months only in 0–7% of treated patients [Hanno *et al.* 2011].

Leppilahti and colleagues administered four weekly intravesical instillations of 40 mg HA after BH in 11 patients and observed a decrease in urinary frequency (less than 75%) and in a visual analogue scale (VAS) pain score (less than 26%) [Leppilahti *et al.* 2002]. Ahmad and colleagues and Shao and colleagues used 40 mg HA administered intravesically to prolong the bladder capacity obtained after BH [Shao *et al.* 2010; Ahmad *et al.* 2008]. They observed in each study an improvement in mean bladder capacity; 492 ml *versus* 776 ml after 16 months of follow up in the 23 patients [Ahmad *et al.* 2008]; +27 ml *versus* −5 ml in the placebo group after 6 months [Shao *et al.* 2010].

In the other six studies the efficacy of HA bladder instillations to reduce symptoms score was assessed. Daha and colleagues evaluated the efficacy of 10 weekly instillations of 40 mg HA in 48 patients with clinical symptoms of IC and a previous positive 0.4 M potassium sensitivity test [Daha *et al.* 2005]. Moreover, they analysed the study population by separating it into two groups: first according to bladder capacity after NaCl cystometry (groups I and II) and then according to bladder capacity reduction rate after a 0.2 M KCl test performed (Ia/IIa $\geq$ 30% and Ib/IIb $<$ 30%). They found symptom relief (VAS scores improvements of 84% and 87%, groups I and II, respectively) more particularly evident in patients with a higher bladder capacity reduction after 0.2 M KCl solution test (mean reduction in VAS scores, 3 in Ia/IIa group *versus* 5 in group Ib/IIb).

Gupta and colleagues showed that 55% of their study population (20/36) improved after six weekly doses of 40 mg HA, particularly those patients with a positive potassium sensitivity test (PST) [Gupta *et al.* 2005].

In a prospective, uncontrolled study, Kallestrup and colleagues reported a 65% of positive response rate after four weekly plus two monthly bladder instillations of 40 mg HA in 20 patients with PBS/IC [Kallestrup *et al.* 2005]. A significant

Table 2. Comparative analysis on studies on interstitial cystitis.

Study	Number of patients	Study design	Median age	Hyaluronic acid dose	Regimen	Follow up (months)	Outcome parameters	Results
Leppilahti *et al.* [2002]	11	PCS	64 (51–76)	40 mg	Four weekly instillations after bladder hydrodistension	NR	VAS pain score Urinary frequency ICAM-1 expression[1]	75% decrease 26% decrease Increased in nonresponders
Daha *et al.* [2005]	48 32 with $C_{max} < 350$ cc (group1) 16 with $C_{max} > 350$ cc (group2)[2]	PCS	54 (22–82)	40 mg	Ten weekly instillations	16	VAS pain score	Improvement 84% group 1 87% group 2 Especially in patients with a C_{max} reduction $\geq$ 30% after 0.2 KCl test
Gupta *et al.* [2005]	38	PCS		40 mg	Six weekly doses and choice to continue monthly in responders		ICSI ICPI	55% of patients improved after six doses (74% and 23% of patients with positive and negative respectively, PST test)
Kallestrup *et al.* [2005]	20	PCS	34–80	40 mg	Four weekly plus two monthly instillations. Choice to continue instillations for responders	36	VAS pain score Urinary frequency Use of analgesic	Reduction in pain score about 2.2-fold after 3 months and 5.2 after 3 years with a decrease of analgesic use Reduction in urinary frequency not observed
Theoharides *et al.* [2008]	252	PCS	(18–69)	10 mg	Oral ingestion four capsules/day	12	VAS score	About 50% reduction
Cervigni *et al.* [2008]	23	PCS	46 (20–65)	40 mg (1.6%) plus chondroitin sulphate (2%)	Weekly for 20 weeks then 2 weeks for 4 weeks and then monthly for 3 months	8	Voiding diary VAS pain score ICSI ICPI PUF	Significant Improvement in all parameters
Riedl *et al.* [2008]	121	PCS	49 (17–83)	40 mg	Weekly instillation until improvement not observed (median instillations for patient 12)		VAS score Quality of life score	Mean VAS score reduction (3.5 posttreatment *versus* 8.5 pretreatment)

(continued)

Table 2. Continued

Study	Number of patients	Study design	Median age	Hyaluronic acid dose	Regimen	Follow up (months)	Outcome parameters	Results
Ahmad *et al.* [2008]	23	CCS	53 (25–81)	40 mg	Two monthly bladder hydrodistension plus hyaluronic acid instillation. Then further treatment depending on initial response (average six treatments in responders)	16	Bladder capacity	Improvement in mean bladder capacity (492 ml *versus* 776 ml)
Shao *et al.* [2010]	47 with functional bladder capacity < 200 ml	RCT	55 (27–76)	40 mg	Four weekly plus two monthly after hydrodistension	9	Mean void per days VAS pain score Bladder capacity after hydrodistension	Hyaluronic acid and heparin might maintain or prolong the effect of hydrodistention in patients with interstitial cystitis

[1]ICAM-1 (intracellular adhesion molecule-1) is one of the identified cell receptors for hyaluronic acid.
[2]*C*max > 350 ml with 0.9% Na solution is not considered a diagnostic criterion for diagnosis of interstitial cystitis.
CCS, case control study; CSI, interstitial cystitis symptom index; ICPI, interstitial cystitis problem index; ICSI, interstitial cystitis symptom index; PCS, prospective cohort study; PST, potassium sensitivity test; PUF, pelvic pain and urgency/frequency symptom scale; RCT, randomized controlled trial; VAS, visual analogue scale.

reduction in pain score was noted (a 2.2-fold decrease in pain score after 3 months and 5.2-fold decrease after 3 years). However, after 3 years of therapy, seven patients (35%) were considered again partial responders.

Theoharides and colleagues used dietary supplements to treat 252 patients affected by PBS/IC in an uncontrolled study. They observed a reduction of about 50% in VAS score over 12 months of drug administration (25 male patients, mean VAS score reduced from 7.6 to 3.4; 227 women patients, mean VAS score reduced from 7.91 to 3.97) [Theoharides *et al.* 2008].

In an open, prospective, unblinded and uncontrolled study Cervigni and colleagues showed an improvement in urinary symptoms after 20 weekly and 3 monthly intravesical instillations of 40 mg HA and CS over 8 months of follow up [Cervigni *et al.* 2008]. They used many questionnaires to evaluate therapy efficacy in 23 women: IC symptom index (ICSI), IC problem index (ICPI), pelvic pain and urgency/frequency (PUF) symptom scale and VAS score for pain–frequency–urgency; they reported a significant improvement in the mean value of each outcome compared with the pretreatment value (ICSI 13 *versus* 11; ICPI 11 *versus* 10; PUF 22 *versus* 17; VAS pain–frequency–urgency 5 *versus* 4, 7 *versus* 5, 6 *versus* 3, respectively).

Similar results were reported by Riedl and colleagues. They administered 40 mg HA weekly in 126 patients with PBS/IC until recording a symptomatic improvement [Riedl *et al.* 2008]. Therefore, the average number of instillations for all patients was 12 and the mean VAS symptom score was reduced to 3.5 *versus* 8.5 from the first treatment.

Finally, although different follow ups and outcomes were used to evaluate treatment efficacy, in each study a therapeutic success was reported. However, some limitations of these studies should be recognized as most were unblinded pilot studies, without a control group. However, the positive results obtained by using HA support the 'domino-like' pathophysiological hypothesis and it warrants further multicentre, randomized, placebo-controlled trial to confirm these early findings. Finally, no serious adverse events were recorded in any of these studies.

Idiopathic detrusor overactivity

The International Continence Society defines idiopathic detrusor overactivity (IDO) as involuntary detrusor contractions during the filling phase, without a clear cause [Abrams *et al.* 2002]. IDO can produce the same storage lower urinary tract symptoms such as frequency, urgency and urge incontinence.

We found only few clinical research and experimental models on GAGs and IDO with conflicting results.

Ferrara observed a higher urinary excretion of GAGs in children affected by enuresis and urinary incontinence than in a control group although in the absence of an urodynamic test [Ferrara *et al.* 2007]. Siracusano and colleagues performed a urodynamic test in their study population (25 women, 63 years median age, with filling lower urinary tract symptoms [LUTS]) and uroflowmetry in the control group (seven men and seven women, 65 years median age, without LUTS) [Siracusano *et al.* 2009].

Although Soler and colleagues concluded in their study that urinary GAGs cannot differentiate urothelial damage from recovery because elevated levels of urinary GAGs can result from either condition [Soler *et al.* 2008], Siracusano and colleagues detected a lower GAGs urinary concentration in LUTS-affected population than in controls and because of this they postulated that a long duration of IDO might generate a chronic subepithelial bladder ischemia, which at the same time damages the epithelium and prevents its regeneration [Siracusano *et al.* 2008].

Unfortunately there is little clinical evidence to establish whether urinary GAGs excretion is low or high, but IDO might be one of the causes of initial epithelium damage and GAGs loss the supporting process.

Nonbacterial cystitis

The bladder is often vulnerable to the adverse effects of drugs because of the frequent excretion of drug metabolites in the urine. The term 'nonbacterial' also includes cystitis caused by pelvic radiation therapy or by adjuvant chemo-immunotherapy for nonmuscle invasive bladder cancer.

As a matter of fact, up to 54% of the patients undergoing intravesical therapy with chemotherapeutic agents to treat superficial bladder

tumours can be affected by nonbacterial cystitis [Drake *et al.* 1998]. Antimicrobials, anticholinergics, anaesthetics and analgesics are often utilized to relieve patients' symptoms but they are not able to prevent the chronic progression of the disease.

Sommariva and colleagues performed a prospective study on 55 male patients (age range 54–81 years) to evaluate the use of 8–24 (depending on the time needed to symptoms' resolution) weekly bladder instillations of 40 mg HA in the treatment of nonbacterial cystitis [Sommariva *et al.* 2010]. Although a control group was not included and over the first 4 weeks 32 mg of dexamethasone were mixed with HA in order to obtain a stronger anti-inflammatory activity, the authors observed an improvement in VAS score (mean initial value 8.6 *versus* 1 at the end of the study) and in bladder capacity (from a mean value of 56 to 276 ml) after 16 weeks.

Samper and colleagues evaluated retrospectively the efficacy of bladder instillations of 40 mg of HA to reduce the vesical toxicity (measured by the Radiation Therapy Oncology Group criteria) induced by radiotherapy [Samper *et al.* 2009]. The study included 95 patients with cervical or endometrial cancer. In 48 of the patients HA was administered 30 min prior to each session of brachytherapy (BT). The authors reported a reduction in acute vesical toxicity after the second session (20.8% HA group *versus* 40.4% without HA group), the fourth session (10.9% HA group *versus* 31.9% without HA group) and whole study period (2.08% HA group *versus* 12.8% without HA group) ($p < 0.05$). No significant differences between groups with regard to the total number of BT sessions, dose per session, total dose or biological equivalent dose were reported.

Urological malignancies

HA is also one of the major matrix molecules in human malignancies [Tammi *et al.* 2008]. It is associated with invasion, lymph angiogenesis, angiogenesis and host–tumour interactions and with local involvement (lymphatic nodes and adjacent organs) and distant metastasis (such as bone) [Sironen *et al.* 2011; Theocharis *et al.* 2010]. HA may be employed also in the diagnosis and treatment of bladder cancer.

HA and hyaluronidase (HAase) have been reported as possible urinary markers to the diagnosis and follow up of bladder cancer. The enzyme-linked immunosorbent assay-like test to dose urinary excretion of HA-HAase is considered promising test for bladder cancer detection for its high sensitivity and specificity (83% and 78% respectively) [Hautmann, 2004].

In some clinical studies HA is attached with some chemotherapeutic agents to make them less lipophilic and so reduce systemic drug absorption and side effects. Recently, Bassi and colleagues evaluated bladder instillation of paclitaxel conjugated with HA for treatment of bladder carcinoma *in situ* refractory to bacillus Calmette–Guérin in a phase I study [Bassi *et al.* 2011]. They did not observe systematic drug absorption or serious adverse events related to drug administration. Although the 60% of study population was disease free at the end of the trial, oncological outcomes did not represent the main endpoint.

Conclusion

Few data are available regarding the mode of action of HA–CS or its effectiveness in the management of bladder diseases. The major issue in interpreting the available evidence regarding HA–CS is that most reported studies are nonrandomized and without a control arm. Thus, this novel therapeutic modality has not been compared with other commonly used therapeutic options in patients with bladder diseases.

HA–CS may be considered for further study, including randomized controlled trials with adequate power. Nevertheless, positive reported findings are very encouraging and may prompt investigators to embark on clinical and experimental trials to better understand the action of GAGs in bladder diseases and to define the role of HA–CS in their management.

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Conflict of interest statement

The authors declare no conflicts of interest in preparing this article.

References

Abrams, P., Cardozo, L., Fall, M., Griffiths, D., Rosier, P., Ulmsten, R. *et al.* (2002) The standardisation of terminology of lower urinary tract function:

report from the standardisation sub-committee of the International Continence Society. *Neurourol Urodyn* 21: 167–178.

Ahmad, I., Sarath Krishna, N. and Meddings, R.N. (2008) Sequential hydrodistension and intravesical instillation of hyaluronic acid under general anaesthesia for treatment of refractory interstitial cystitis: a pilot study. *Int Urogynecol J Pelvic Floor Dysfunct* 19: 543–546.

Arms, L. and Vizzard, M.A. (2011) Neuropeptides in lower urinary tract function. *Handb Exp Pharmacol* 202: 395–423.

Bassi, P.F., Volpe, A., D'Agostino, D., Palermo, G., Renier, D., Franchini, S.M. *et al.* (2011) Paclitaxel-hyaluronic acid for intravesical therapy of bacillus Calmette–Guérin refractory carcinoma *in situ* of the bladder: results of a phase I study. *J Urol* 185: 445–449.

Cervigni, M., Natale, F., Nasta, L., Padoa, A., Voi, R.L. and Porru, D. (2008) A combined intravesical therapy with hyaluronic acid and chondroitin for refractory painful bladder syndrome/interstitial cystitis. *Int Urogynecol J Pelvic Floor Dysfunct* 19: 943–947.

Clemens, J.Q. (2010) Afferent neurourology: a novel paradigm. *Neurourol Urodyn* 29(Suppl. 1): S29–S31.

Constantinides, C., Manousakas, T., Nikolopoulos, P., Stanitsas, A., Haritopoulos, K. and Giannopoulos, A. (2004) Prevention of recurrent bacterial cystitis by intravesical administration of hyaluronic acid: a pilot study. *BJU Int* 93: 1262–1266.

Daha, L.K., Riedl, C.R., Lazar, D., Hohlbrugger, G. and Pflüger, H. (2005) Do cystometric findings predict the results of intravesical hyaluronic acid in women with interstitial cystitis? *Eur Urol* 47: 393–397.

Damiano, R., Quarto, G., Bava, I., Ucciero, G., Palumbo, M.I., Autorino, R. *et al.* (2011) Prevention of recurrent urinary tract infections by intravesical administration of hyaluronic acid and chondroitin sulphate: a placebo-controlled randomised trial. *Eur Urol* 59: 645–651.

Drake, M.J., Nixon, P.M. and Crew, J.P. (1998) Drug-induced bladder and urinary disorders. Incidence, prevention and management. *Drug Saf* 19: 45–55.

Ferrara, P., Marrone, G., Mastrangelo, A., Nicoletti, A., Emmanuele, V. and Fasano, A. (2007) Increased excretion of glycosaminoglycans in children with urinary incontinence compared to those with monosymptomatic nocturnal enuresis. *Scand J Urol Nephrol* 41: 218–222.

Foxman, B. (2002) Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. *Am J Med* 113(Suppl. 1A): 5S–13S.

Foxman, B., Gillespie, B., Koopman, J., Zhang, L., Palin, K., Tallman, P. *et al.* (2000) Risk factors for second urinary tract infection among college women. *Am J Epidemiol* 51: 1194–1205.

Geppetti, P., Nassini, R., Materazzi, S. and Benemei, S. (2008) The concept of neurogenic inflammation. *BJU Int* 101(Suppl. 3): 2–6.

Gupta, S.K., Pidcock, L. and Parr, N.J. (2005) The potassium sensitivity test: a predictor of treatment response in interstitial cystitis. *BJU Int* 96: 1063–1066.

Hanno, P.M., Burks, D.A., Clemens, J.K., Dmochowski, R.R., Ericksen, D., Fitzgerald, M.P. *et al.* (2011) Cystoscopy under anesthesia with hydrodistension. In: Diagnosis and Treatment of Interstitial Cystitis/Bladder Pain Syndrome. AUA Guidelines, American Urological Association: Linthicum, MD.

Hautmann, S., Toma, M., Lorenzo Gomez, M.F., Friedrich, M.G., Jaekel, T., Michl, U., Schroeder, G.L. *et al.* (2004) Immunocyt and the HA-HAase urine tests for the detection of bladder cancer: a side-by-side comparison. *Eur Urol* 46: 466–471.

Iavazzo, C., Athanasiou, S., Pitsouni, E. and Falagas, M.E. (2007) Hyaluronic acid: an effective alternative treatment of interstitial cystitis, recurrent urinary tract infections, and hemorrhagic cystitis? *Eur Urol* 51: 1534–1541.

Jones, C.A. and Nyberg, L. (1997) Epidemiology of interstitial cystitis. *Urology* 49(Suppl. 5A): 2–9.

Kallestrup, E.B., Jorgensen, S.S., Nordling, J. and Hald, T. (2005) Treatment of interstitial cystitis with Cystistat: a hyaluronic acid product. *Scand J Urol Nephrol* 39: 143–147.

Kanai, A.J. (2011) Afferent mechanism in the urinary tract. *Handb Exp Pharmacol* 202: 171–205.

Leppilahti, M., Hellström, P. and Tammela, T.L. (2002) Effect of diagnostic hydrodistension and four intravesical hyaluronic acid instillations on bladder ICAM-1 intensity and association of ICAM-1 intensity with clinical response in patients with interstitial cystitis. *Urology* 60: 46–51.

Lilly, J.D. and Parsons, C.L. (1990) Bladder surface glycosaminoglycans is a human epithelial permeability barrier. *Surg Gynecol Obstet* 171: 493–496.

Lipovac, M., Kurz, C., Reithmayr, F., Verhoeven, H.C., Huber, J.C. and Imhof, M. (2007) Prevention of recurrent bacterial urinary tract infections by intravesical instillation of hyaluronic acid. *Int J Gynaecol Obstet* 96: 192–195.

Naber, K.G., Bergman, B., Bishop, M.C., Bjerklund, T.E., Botto, H., Lobel, B. et al. (2001) EAU guidelines for the management of urinary and male genital tract infections. Urinary Tract Infection (UTI) Working Group of the Health Care Office (HCO) of the European Association of Urology (EAU). *Eur Urol* 40: 576–588.

Parsons, C.L. (1994) The therapeutic role of sulfated polysaccharides in urinary bladder. *Urol Clin North Am* 21: 93–100.

Parsons, C.L. (2011) The role of a leaky epithelium and potassium in the generation of bladder symptoms in interstitial cystitis/overactive bladder, urethral syndrome, prostatitis and gynaecological chronic pelvic pain. *BJU Int* 107: 370–375.

Riedl, C.R., Engelhardt, P.F., Daha, K.L., Morakis, N. and Pflüger, H. (2008) Hyaluronan treatment of interstitial cystitis/painful bladder syndrome. *Int Urogynecol J Pelvic Floor Dysfunct* 19: 717–721.

Samper Ots, P.M., Carrizosa, C.L., Rodriguez, A., de Dios Saez, J., Delgado, J.M., de Miguel, M.M. *et al.* (2009) Vesical instillations of hyaluronic acid to reduce the acute vesical toxicity caused by high-dose brachytherapy do not affect the survival: a five-year follow-up study. *Clin Transl Oncol* 11: 828–834.

Shao, Y., Shen, Z.J., Rui, W.B. and Zhou, W.L. (2010) Intravesical instillation of hyaluronic acid prolonged the effect of bladder hydrodistention in patients with severe interstitial cystitis. *Urology* 75: 547–550.

Siracusano, S., Cucchi, A., Ciciliato, S., Lampropoulou, N. and Vittur, F. (2009) Urinary levels of glycosaminoglycans in patients with idiopathic detrusor overactivity. *Int Urogynecol J Pelvic Floor Dysfunct* 20: 1477–1480.

Sironen, R.K., Tammi, M., Tammi, R., Auvinen, P.K., Anttila, M. and Kosma, V.M. (2011) Hyaluronan in human malignancies. *Exp Cell Res* 15: 383–391.

Soler, R., Bruschini, H., Martins, J.R., Dreyfuss, J.L., Camara, N.O., Alves, M.T. *et al.* (2008) Urinary glycosaminoglycans as biomarker for urothelial injury: is it possible to discriminate damage from recovery? *Urology* 72: 937–942.

Sommariva, M.L., Sandri, S.D. and Guerrer, C.S. (2010) Treatment of acute iatrogenic cystitis secondary to bladder chemo-immuno-instillation or pelvic radiotherapy. *Urologia* 77: 187–192.

Tammi, R.H., Kultti, A., Kosma, V.M., Pirinen, R., Auvinen, P. and Tammi, M.I. (2008) Hyaluronan in human tumors: pathobiological and prognostic messages from cell-associated and stromal hyaluronan. *Semin Cancer Biol* 18: 288–295.

Theocharis, A.D., Skandalis, S.S., Tzanakakis, G.N. and Karamanos, N.K. (2010) Proteoglycans in health and disease: novel roles for proteoglycans in malignancy and their pharmacological targeting. *FEBS J* 277: 3904–3923.

Theoharides, T.C., Kempuraj, D., Vakali, S. and Sant, G.R. (2008) Treatment of refractory interstitial cystitis/painful bladder syndrome with CystoProtek – an oral multi-agent natural supplement. *Can J Urol* 15: 4410–4414.

van de Merwe, J.P., Nordling, J., Bouchelouche, P., Bouchelouche, K., Cervigni, M., Daha, L.K. *et al.* (2008) Diagnostic criteria, classification, and nomenclature for painful bladder syndrome/interstitial cystitis: an ESSIC proposal. *Eur Urol* 53: 60–67.